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Germline *PKHD1* mutations are protective against colorectal cancer

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Abstract

The autosomal recessive polycystic kidney disease (ARPKD) gene, *PKHD1*, has been implicated in the genesis or growth of colorectal adenocarcinoma, as a high level of somatic mutations were found in colorectal tumor tissue. To determine whether carriers of a single *PKHD1* mutation are at increased risk of colorectal carcinoma, we assessed the prevalence of the commonest European mutation, T36M. First, we assayed a European cohort of ARPKD patients and found T36M was responsible for 13.1% of mutations. We then, investigated two European cohorts with colorectal adenocarcinoma vs two control cohorts of similar age and gender. Screening for the most common *PKHD1* mutation, T36M, we detected 15:3603 (0.42%) controls vs 1:3767 (0.027%) colorectal cancer individuals, indicating that heterozygous *PKHD1* mutations are not a risk factor and are protective (p=0.0002). We also show that the carriage rate for *PKHD1* mutations in the European population is higher than previous accepted at 3.2% (1:31 genomes).

Keywords

Autosomal Recessive Polycystic Kidney Disease; CANcer gene; Polycystic Kidney Hepatic Disease Gene 1; Colon cancer

INTRODUCTION

Autosomal recessive polycystic kidney disease (ARPKD) is characterized by dilation of collecting ducts (CDs) in the kidney and hepatic fibrosis with or without non-obstructive biliary dilatation. ARPKD most often presents *in utero* or at birth with the finding of marked renal enlargement, due to cysts, and biliary dysgenesis. The disease is characterized by loss of epithelial polarity and dedifferentiation of the cells lining the biliary tree and collecting

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duct of the kidney. ARPKD is due to mutations in the polycystic kidney and hepatic disease 1 (*PKHD1*) gene and is recessive with heterozygote `carriers' being phenotypically normal. *PKHD1* has also been implicated in the genesis or growth of colon adenocarcinoma. In a high throughput screen of 14,661 human protein coding transcripts, *PKHD1* was found to be the seventh most common somatically mutated gene in colorectal cancer cases(Sjoblom et al. 2006). There has been debate on whether some of the genes described as candidate cancer genes (CAN) in the above study are truly mutated above background levels. *PKHD1* initially appeared to be a CAN gene (CAN score 3.5), but in the face of more stringent criteria failed to reach statistical significance(Chng 2007; Forrest and Cavet 2007; Getz et al. 2007; Loeb and Bielas 2007; Rubin and Green 2007). However, the finding that a gene known to be involved in the control of epithelial differentiation was somatically mutated in 5 of 35 colon tumors (14.2%) is of interest. A prediction from this study is that individuals carrying a germline *PKHD1* mutation are at enhanced risk of colorectal cancer. In the European derived population, T36M is the most common mutant allele (always a 107 C → T transition(Ward et al. 2002)) is responsible for approximately 17.6% of all *PKHD1* mutations(Bergmann et al. 2005); other described mutations are individually rare or only more common in specific populations(Consugar et al. 2005; Rossetti et al. 2003). Homozygotes with the M36 allele genotype uniformly develop ARPKD which may be severe, implying that this allele significantly disrupts the normal function of the ARPKD protein, fibrocystin(Rossetti et al. 2003). ARPKD affects about 1:20,000 neonates (this is an approximate and conservative estimate, with incidences of 1:40,000 quoted elsewhere(Zerres et al. 1984)) and, thus, a carrier of 1:70 has been suggested for genetic counseling. On this basis, the T36M allele is predicted to be present in about 1:412 European genomes. This presents a testable hypothesis, whether T36M carriers have an increased risk of colorectal carcinoma. A prevalence of 1:412 is within the range that can be assessed in our large colorectal cancer cohort. Since the precise carrier frequency of *PKHD1* mutations is unknown we also used this study to better estimate the frequency of all *PKHD1* mutations in Europeans.

RESULTS

Initially, we developed a TaqMan assay (Applied Biosystems, Foster City, CA) for the T36M allele and assessed its prevalence in a cohort of European derived ARPKD patients. This served to both validate the assay and to supply a baseline T36M prevalence rate in an ARPKD patient cohort, a sample of individuals with classical ARPKD and their parents(Rossetti et al. 2003). In our cohort, we derived an allele frequency of 13.1%; 95% CI: 8.9–18.8%. The total number of alleles is an odd number, because in one case we only had one parent and no DNA from an affected individual. The 95% confidence level overlaps with the 17.6% incidence rate observed by others(Bergmann et al. 2005). All mutations were confirmed by sequencing.

We next screened 1842 subjects with a history of sporadic colorectal adenocarcinoma (US residents of European origin) and 1601 clinic-based control subjects (similar in age, gender and state of residence to the cancer cohort), see Table 1. Six T36M heterozygotes were found in the control sample (0.37%), and none (0.0%) in the colorectal cancer sample (p=0.01 Fisher's exact test).

To confirm the initial study, which showed an unexpected protective effect of T36M on colorectal cancer, we assembled a new cohort of control and colorectal cancer patients (of European origin) with no overlap with the initial sample. In this second study, we found nine T36M heterozygotes in the control sample (0.45%, n=2002 subjects) and one in the colorectal cancer sample (0.05%, n=1925 subjects) (p=0.022). This individual was confirmed to have stage 3 colorectal carcinoma. Together, the data show that the prevalence

of T36M is 15:3603 (0.42%) in the control and 1:3767 (0.027%) in the colorectal sample (p=0.0002) (Table 1). This indicates that the T36M allele is not associated with an increased risk of colon cancer; indeed, the opposite hypothesis that the T36M mutation is protective becomes extremely significant, with an odds ratio =0.072, 95% CI: 0.003–0.36.

To calculate the carriage rate of all *PKHD1* mutations in the European sample, we used the allelic incidence of T36M of 1:480 alleles (0.42% of genomes) in controls and the incidence of T36M in our ARPKD sample, (1 in 7.6 *PKHD1* mutations are T36M (13.1%)), to project a 3.2% (7.6 × 0.42), 1:31 carrier frequency for *PKHD1* in the European population (3.2%, 95% CI:1.7–5.9%). The previous estimate of ARPKD mutation carrier rate is 1:70 or 1.43%, which is just outside the 95% confidence limits of our study. These sixteen T36M heterozygotes were confirmed by Dye-terminator DNA sequencing.

DISCUSSION

PKHD1 has been implicated in the genesis of colorectal carcinoma by high throughput sequencing of 120, 839 coding exons of the human genome in normal somatic tissue and colorectal tumors. This screen detected mutations in the known CAN genes, *APC* (CAN score > 10), *KRAS* (>10), *TP53* (>10), *FBXW7* (5.1), *SMAD4* (4.6) and *MLL3* (3.7), all of which are tumor suppressors or proteins activated in carcinogenesis. *PKHD1* has a CAN score of 3.5, tied as the 7th highest CAN score with *GUCY1A2* and *EPHB6*. Based on these data, we predicted that *PKHD1* mutations would enhance susceptibility to colorectal carcinoma. However our data appears to run counter to this and suggests that *PKHD1* mutation carriers may be protected from colorectal cancer. One possible hypothesis to explain the findings is that reduction of fibrocystin activity might result in enhanced mitotic instability which paradoxically inhibits carcinogenesis. It has recently been shown that fibrocystin localizes to the centrosome in a range of kidney cell lines and that depletion of fibrocystin in IMCD3 and MDCK results in centrosome amplification, chromosome lagging and multipolar spindle formation. Centrosome amplification was also observed in ARPKD kidneys(Zhang et al. 2010). Such alterations in karyotypic stability would be predicted to be oncogenic. However, mutations in genes involved in mitotic segregation of chromosomes, but which have no role in DNA metabolism or repair (such as the centromere-linked protein CENP-E), induce aneuploidy without DNA damage and have a complex effect on tumor development. *Cenp*$^{+/-}$ mice with pure aneuploidy develop lymphomas and lung tumors at an enhanced rate in aged mice; however, the same genotype can suppress chemically or genetically induced tumor formation(Weaver et al. 2007). It may be that germline mutations in *PKHD1* act similarly, inducing susceptibility to centrosomal amplification in certain contexts, so that pre-malignant cells have too much genomic instability to form aggressive tumors (undergoing mitotic catastrophe and apoptosis), accounting for the protective effect of the T36M *PKHD1* mutation for the development of colorectal carcinoma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Incidence of T36M change in the ARPKD cohort, a cohort with proven colorectal carcinoma and a clinic-based control group without a history of colonic carcinoma.

	ARPKD cohort	Controls Group 1	Colorectal Carcinoma Group 1
36M alleles	24	6	0
Total alleles (subjects)	183 (92)	3202 (1601)	3684 (1842)
36M rate per allele (95% CI)	13.1% (8.9–18.8%)	0.19% (0.088–0.41%)	0% (0.0007–0.1%)
36M rate per subject	26.1%	0.37%**	0.0%**

	Controls Group2	Colorectal Carcinoma group 2	Control Combined	Colorectal Combined
36M alleles	9	1	15	1
Total alleles(subject)	4004 (2002)	3850 (1925)	7206 (3603)	7534 (3767)
36M rate per allele (95% CI)	0.22% (0.11–0.45%)	0.026% ($1.35e^{-3}$–0.17%)	0.21% (0.12–0.35%)	0.013% ($6.9e^{-4}$–0.086%)
36M rate per subject	0.45%*	0.05%*	0.42%***	0.03%***

** The difference between the control and cancer sample is significant at p=0.01 (Fisher's exact test).

* The difference between the control and cancer sample is significant at p=0.02 (Fisher's exact test).

*** The difference between the combined control and cancer sample is significant at p=0.0002 (Fisher's exact test). Odd ratio =0.072, 95% CI: 0.003–0.36.